Fluidigm

Using the C1™ Single-Cell Auto Prep System to Capture Cells from Cell Culture and Perform Preamplification Using TaqMan® Assays

PN 100-6117 A1

Protocol

Contents

Introduction

This protocol allows the user to capture cells and perform target preamplification using the Fluidigm C_1™ Single-Cell Auto Prep System and C_1 Single-Cell Auto Prep Array Integrated Fluidic Circuits (IFCs). This protocol provides an in-depth explanation for all steps performed, including: capturing cells, staining for viability, imaging cells, lysing cells, performing reverse transcription and preamplification, and finally, harvesting the amplified products. It describes the procedure to evaluate the RNA content of cells and to harvest the products generated from a C_1 Single-Cell Auto Prep Array IFC. Gene expression analysis of preamplified amplicons is then performed with 48.48 or 96.96 Dynamic Array™ IFCs using the BioMark™ or BioMark HD System.

Preamplification enriches samples for loci of interest, maintains relative abundance between loci, and permits quantitative C_q information to be derived. Quantitative PCR is then performed. See Devonshire *et al*. *BMC Genomics* 2011, 12.118, for more information on preamplification.

Figure 1 below shows the preamplification process using the Single Cell-to-CT™ Kit (Ambion/Life Technologies).

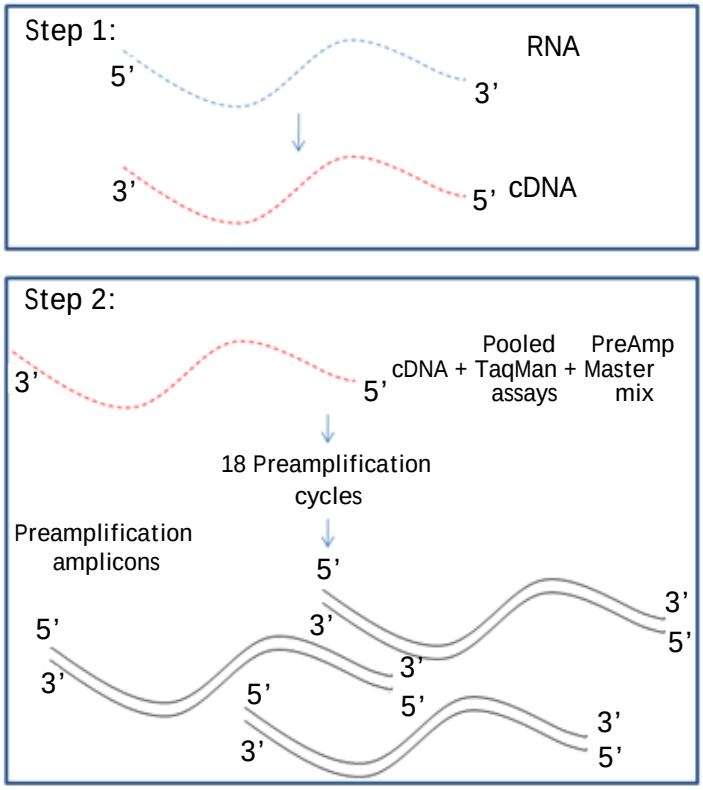


Figure 1 Overview of the preamplification protocol with TaqMan assays

The optional live/dead cell staining step uses the LIVE/DEAD® Viability/Cytotoxicity Kit, which tests the viability of a cell based on the integrity of the cell membrane. This test contains two chemical dyes. The first dye is green-fluorescent calcein-AM, which stains live cells. This dye is cell permeable and tests for active esterase activity in live cells. The second dye is red-fluorescent ethidium homodimer-1, which will only stain cells if the integrity of the cell membrane has been lost.

Overview of Experimental Workflow

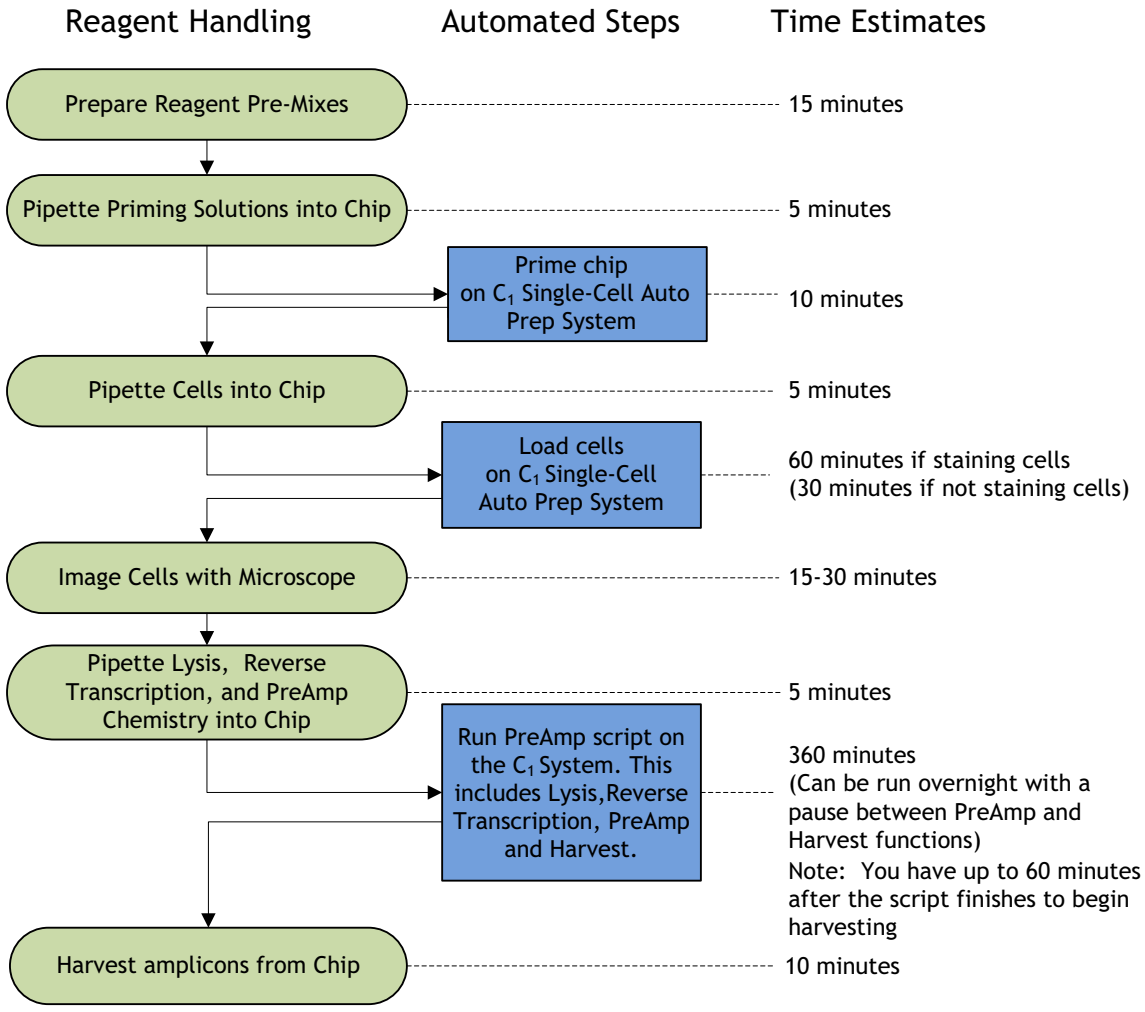


Figure 2 Overview of the cell capture procedure

References

1 *Fluidigm® BioMark™ HD Data Collection Software User Guide* (PN 100-2451)
2 *Fluidigm® Real-Time PCR Analysis Software User Guide* (PN 68000088)
3 *Fluidigm® C_1 Single-Cell Auto Prep System User Guide* (PN 100-4977)
4 *Fluidigm® Application Guidance: Single-Cell Analysis* (PN 100-5066)
5 LIVE/DEAD® Cell Viability/Cytotoxicity Kit for mammalian cells (Invitrogen, PN MP03224)
6 *Minimum Specifications for Single-Cell Imaging* (Fluidigm, PN 100-5004)
7 iNCyto Disposable Hemocytometer www.incyto.com/product/product02_detail.php

Required Reagents

Stored at -20 °C

- Single Cell-to-CT™ Kit (Ambion, PN 4458237)
- C_1 Single-Cell Auto Prep Module 2 Kit (Fluidigm, PN 100-5519)
  - C_1 Loading Reagent (Fluidigm)
  - C_1 DNA Dilution Reagent (Fluidigm)
  - C_1 Harvest Reagent (Fluidigm)
  - C_1 Preloading Reagent (Fluidigm)
  - C_1 Lysis Plus Reagent (Fluidigm)
- (Optional) LIVE/DEAD Cell Viability/Cytotoxicity Kit (Invitrogen, PN MP03224)
- 20X TaqMan® Gene Expression Assays 18 µM each

Stored at 4 ºC

- C_1 Single-Cell Auto Prep Module 1 Kit (Fluidigm, PN 100-5518)
  - C_1 Blocking Reagent (Fluidigm)
  - C_1 Suspension Reagent (Fluidigm)

Stored at room temperature

- C_1 Cell Wash Buffer (Fluidigm) (also from C_1 Module 1 Kit)
- Low lint cloth (Fluidigm)
- 70% Ethanol in a squirt bottle
- DNA-free Water, Teknova
- (Optional) SuperNase-IN™ (Ambion, PN AM2694)

- (Optional) iNCyto C-Chip Disposable Hemocytometer (Neubauer Improved, PN DHC-N01)

C_1 Single-Cell Auto Prep Module 1 Kit 100-5518

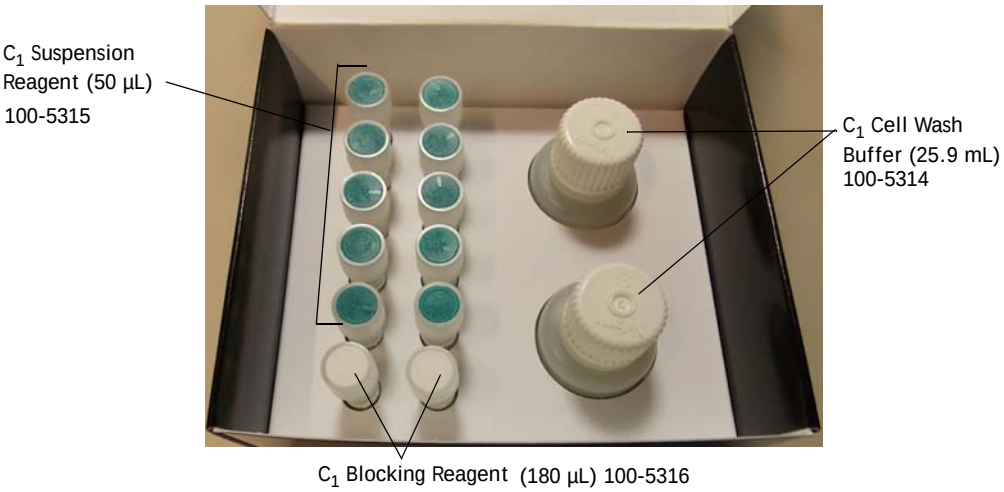


Figure 3 C_1 Single-Cell Auto Prep Module 1 Kit

IMPORTANT: Store the C_1 Single-Cell Auto Prep Module 1 Kit at 4 ºC as soon as it is received.

C_1 Single-Cell Auto Prep Module 2 Kit 100-5519

Figure 4 C_1 Single-Cell Auto Prep Module 2 Kit

IMPORTANT: Store the C_1 Single-Cell Auto Prep, Module 2 kit in a -20 ºC freezer as soon as it is received.

Required Equipment

- C_1 Single-Cell Auto Prep System
- C_1 Single-Cell Auto Prep Array IFCs (barcode 1782 or 1783)
- Suggested: 2 biocontainment hoods to prevent DNA contamination of lab and samples
- 96-well PCR Plate (USA Scientific, TempPlate™ semi-skirted, PN 1402-9700)
- 2 centrifuges: 1 for Eppendorf tubes, 1 for 96-well plates
- Vortex
- Suggested: Imaging equipment compatible with C_1 Auto Prep Array integrated fluidic circuits (IFCs). (See *Minimum Specifications for Single-Cell Imaging*, PN 100-5004, for more detail.)

Chip Types and Related Scripts

There are currently two C_1 System-compatible IFCs for medium and large single cells. See table below for more information.

Cell Size	Barcode (prefix)	Script Names	Description
Medium (10-17 µm)	1782x	Prime (1782x)	Primes the control line and cell capture channels of the 10-17 µm PreAmp array (1782x)
		Cell Load (1782x)	Cell loading and washing for PreAmp of 10-17 µm diameter cells (1782x)
		Cell Load & Stain (1782x)	Cell loading, staining, and washing for PreAmp of 10-17 µm diameter cells (1782x)
		PreAmp (1782x)	Loading, thermal, and harvest protocol for single-cell lysis, reverse transcription, and cDNA preamplification for the 10-17 µm PreAmp array (1782x)
Large (17-25 µm)	1783x	Prime (1783x)	Primes the control line and cell capture channels of the 17-25 µm PreAmp array (1783x)
		Cell Load (1783x)	Cell loading and washing for PreAmp of 17-25 µm diameter cells (1783x)
		Cell Load & Stain (1783x)	Cell loading, staining, and washing for PreAmp of 17-25 µm diameter cells (1783x)
		PreAmp (1783x)	Loading, thermal, and harvest protocol for single-cell lysis, reverse transcription, and cDNA preamplification for the 17-25 µm PreAmp array (1783x)

Table 1 IFCs and related scripts

Safety

It is the individual's responsibility to review all MSDSs for chemicals used in this procedure before running the test.

As with all procedures, the following general safety guidelines apply:

- Personal Protective Equipment (PPE): safety glasses, fully-enclosed shoes, gloves
- Know the locations of all safety equipment (fire extinguishers, spill kits, eyewashes/showers, first aid kits, material safety data sheets, etc.), the emergency exit locations, and emergency/injury reporting procedures
- No eating, drinking, or smoking in lab areas
- Maintain clean work areas
- Wash hands before leaving the lab

HOT SURFACE! The C_1 Single-Cell Auto Prep System thermal cycler chuck gets hot and can burn your skin. Please use caution when working near the chuck.

PINCH HAZARD! The C_1 Single-Cell Auto Prep System door and shuttle can pinch your hand. Please make sure your fingers, hand, shirt sleeve, etc. are clear of the door and shuttle when loading or ejecting a chip.

BIOHAZARD! If you are putting live cells on the C_1 Single-Cell Auto Prep System, please use personal protective equipment and your lab's safety protocol to limit biohazard risks.

Reagent Mixes

Overview – Premixes to prepare

The following instructions prepare reagents sufficient for one chip. The 180 nM Pooled Primer Mix can be made in advance and stored. All other reagents can be scaled up if running multiple chips simultaneously.

NOTE: Allow C_1 Cell Wash Buffer, C_1 DNA Dilution Reagent and C_1 Harvest Reagent (Fluidigm) to equilibrate to room temperature prior to use.

1. Pooled TaqMan Primers (180nM)
2. Lysis Final Mix
3. Reverse Transcription (RT) Final Mix
4. PreAmp Final Mix
5. Optional LIVE/DEAD Cell Staining Solution

Reagent Retrieval from Storage/Freezers

	Required Reagents	Preparation	Kit Name
1. Pooled TaqMan Primers (180 nM)	TaqMan 20X stocks (18 µM)	Remove from -20 ºC freezer and thaw to room temperature in a DNA-free hood	TaqMan gene expression primer stocks (Life Technologies)
	C_1 DNA Dilution Reagent	Remove from -20 ºC freezer and thaw to room temperature in a DNA-free hood	Fluidigm C_1 Module 2 Kit
2. Lysis Final Mix	Single-Cell Lysis Solution	Remove from -20 ºC freezer, thaw, and keep on ice	Single Cell-to-CT Kit (Ambion)
	C_1 Lysis Plus Reagent	Remove from -20 ºC freezer and thaw to room temperature	Fluidigm C_1 Module 2 Kit
3. RT Final Mix	Stop Solution	Remove from -20 ºC freezer, thaw, and keep on ice	Single Cell-to-CT Kit
	Single Cell VILO RT	Remove from -20 ºC freezer, thaw, and keep on ice	Single Cell-to-CT Kit
	Single Cell Super Script RT	Remove from -20 ºC freezer, thaw, and keep on ice	Single Cell-to-CT Kit
	C_1 Loading Reagent	Remove from -20 ºC freezer and thaw to room temperature	Fluidigm C_1 Module 2 Kit
4. PreAmp Final Mix	Single-Cell PreAmp Mix	Remove from -20 ºC freezer, thaw, and keep on ice	Single Cell-to-CT Kit
	Water	Remove from room temperature storage	Teknova
5. Optional LIVE/ DEAD Cell Staining	C_1 Cell Wash Buffer	Remove from room temperature storage area	Fluidigm C_1 Module 1 Kit
	Ethidium homodimer-1	Remove from -20 ºC freezer and keep in the dark as much as possible	LIVE/DEAD Cell Viability/ Cytotoxicity Kit (Invitrogen)
	Calcein AM	Remove from -20 ºC freezer and keep in the dark as much as possible	LIVE/DEAD Cell Viability/ Cytotoxicity Kit (Invitrogen)
6. Priming	C_1 Blocking Reagent	Remove from 4 ºC freezer and thaw to room temperature	Fluidigm C_1 Module 1 Kit
	C_1 Preloading Reagent	Remove from-20 ºC freezer and thaw to room temperature	Fluidigm C_1 Module 2 Kit
7. Cell Loading	C_1 Suspension Reagent	Remove from 4 ºC freezer and thaw to room temperature	Fluidigm C_1 Module 1 Kit

Table 2 Reagent supplies

Pooled TaqMan® Primers (180 nM)

TaqMan gene expression assays are provided as 20X forward and reverse primer and probe mixes with each primer at a concentration of 18 µM.

1. After 20X TaqMan gene expression assays are thawed, vortex and centrifuge at 2,000 rpm for 1 minute.
2. In a DNA-free hood, make the Pooled Primers by combining equal volumes of each 20X TaqMan assay (18 µM primer pair), followed by adding C_1 DNA Dilution Reagent to reach a final concentration of 180 nM per primer. Vortex for 5 seconds and spin briefly to collect contents. Keep on ice until use.

Components	Volume
1 µL each primer pair (20X TaqMan assays)	1.0 µL (x 96 = 96 µL)
C_1 DNA Dilution Reagent (Fluidigm) (30 mL bottle)	4.0 µL
Total	**100.0 µL**

Table 3 Pooled primers (180 nM)

NOTE: The Pooled Primers (180 nM) can be made in advance and pre-aliquoted prior to use. The mix pool can be stored for up to six months at -20 ºC.

Lysis Final Mix

1. Once the Single-Cell Lysis Solution from the Single Cell-to-CT™ Kit is thawed, vortex for 3 seconds and spin briefly to collect contents.
2. Prepare 18.0 µL of Lysis Final Mix by combining the following components.

Components	Volume (µL)
Single-Cell Lysis Solution (Ambion)	12.75
C_1 Lysis Plus Reagent (Fluidigm)	4.35
C_1 DNA Dilution Reagent (Fluidigm) (30 mL bottle)	0.9
Total	**18.0**

Table 4 Lysis Final Mix

3. Vortex for 5 seconds and spin briefly to collect contents. Keep on ice until use.

RT Final Mix

1. Once the Stop Solution and Single Cell VILO RT Mix from the Single Cell-to-CT Kit are thawed, vortex each for 3 seconds and spin briefly to collect contents.
2. In a DNA-free hood, prepare 12 µL of RT (Reverse Transcription) Final Mix by mixing according to the table below. Vortex briefly and spin briefly to collect contents. Keep on ice until use.

Components	Volume (µL)
Stop solution (Ambion)	1.94
Single-Cell VILO RT Mix (Ambion)	5.84
Single-Cell SuperScript RT (Ambion)	3.62
C_1 Loading Reagent (Fluidigm)	0.6
Total	**12.0**

Table 5 RT Final Mix

PreAmp Final Mix

1. In a DNA-free hood, prepare 60 µL of PreAmp Final Mix according to the table below. Briefly vortex and spin to collect contents before use. Keep on ice until ready to use.

Components	Volume (µL)
Single-Cell PreAmp Mix (Ambion)	12.0
C_1 Loading Reagent (Fluidigm)	3.0
Pooled Primers (180 nM)	15.0
DNA-Free Water	30.0
Total	**60.0**

Table 6 PreAmp Final Mix

Optional: Preparing LIVE/DEAD Cell Staining Solution

NOTE: Keep the dye tubes closed and in the dark as much as possible as they can hydrolyze over time. When not in use, store in airtight bag with desiccant pack at -20 ºC.

NOTE: Cell staining solution may be prepared up to 2 hours in advance of loading onto the C_1 chip. Keep on ice before pipetting into chip.

1. Vortex the dyes well before pipetting.
2. Prepare the LIVE/DEAD stain according to table below.

3 Vortex the C_1 LIVE/DEAD staining solution well before pipetting onto chip.

Components	Volume
C_1 Cell Wash Buffer (Fluidigm) (30 mL bottle)	1.25 mL
Ethidium homodimer-1 (LIVE/DEAD kit, Invitrogen/Molecular Probes)	2.5 µL
Calcein AM (LIVE/DEAD kit, Invitrogen/ Molecular Probes)	0.625 µL

Table 7 Staining solution

Running a C_1 Chip

NOTE: When pipetting into the C_1 chip, always stop at the first stop on the pipette to avoid creating bubbles in the inlets. If a bubble is introduced, ensure that it floats to the top of the well.

NOTE: Vortex and spin all reagent mixes before pipetting into the chip.

Using the Chip Map Loading Plate

A black chip map loading plate accessory can be used to assist chip pipetting.

1 Place C_1 Single-Cell Auto Prep Array IFC onto the map.

2 Pipette reagents according to the following instructions.

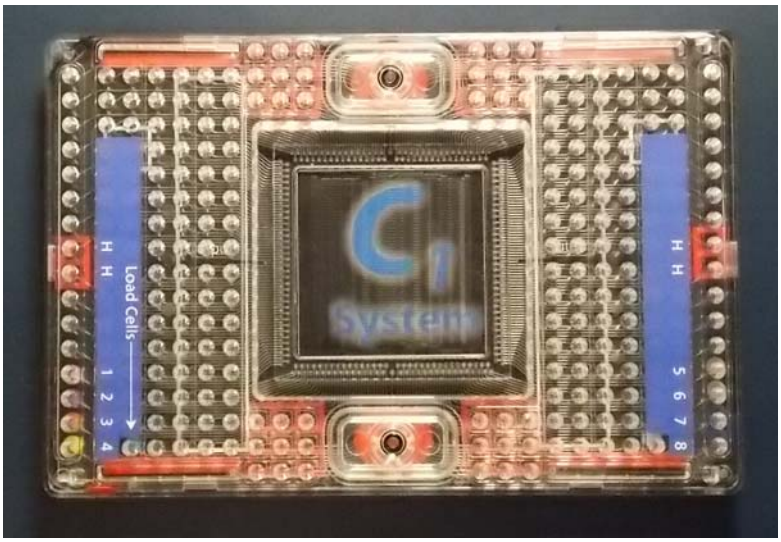


Figure 5 IFC on chip map loading plate

Priming the Chip

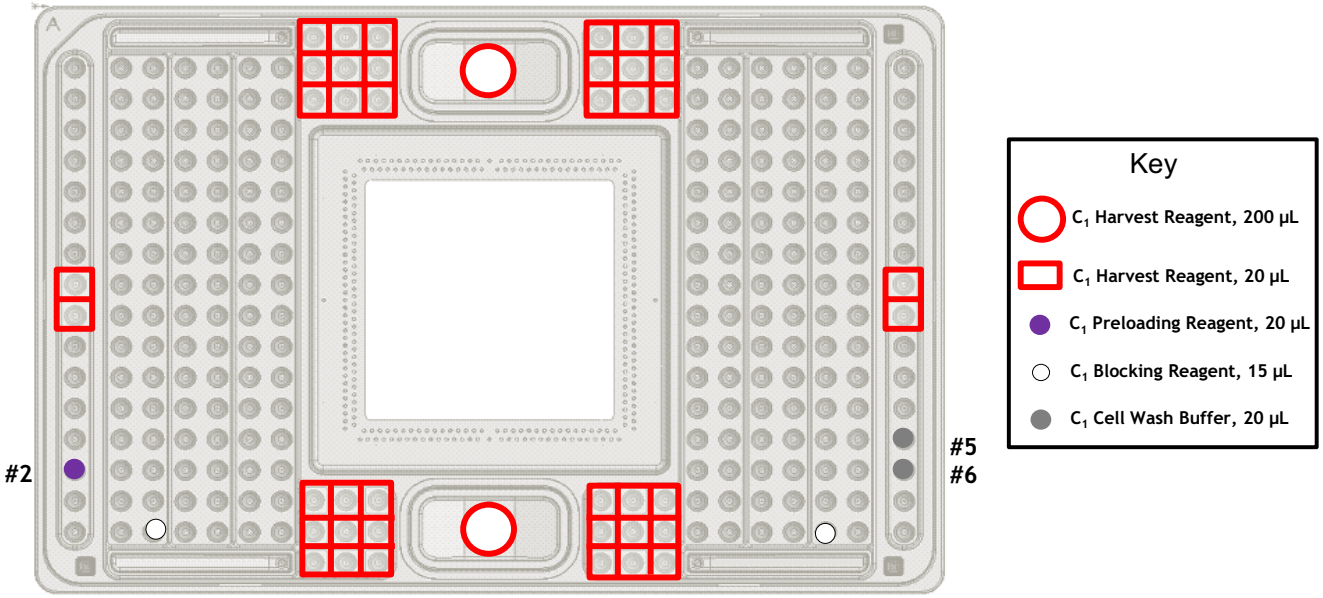


Figure 6 C_1 Chip priming pipetting map

1. Add 200 µL of C_1 Harvest Reagent from 4 mL bottle into accumulators marked with red circles in Figure 6 using a pipette tip.
2. Pipette 20 µL of C_1 Harvest Reagent into wells marked with red squares on each side of the accumulators (36 total).
3. Pipette 20 µL of C_1 Harvest Reagent into the two wells on each side of the chip in the middle of the outside columns of wells marked with red squares. These wells are marked on the bottom of the chip with a notch to ensure they are easily located.
4. Pipette 20 µL of C_1 Preloading Reagent into inlet 2 marked with a purple dot.
5. Pipette 15 µL of C_1 Blocking Reagent into the Cell Inlet and Outlet marked with white dots.
6. Pipette 20 µL C_1 Cell Wash Buffer (from 30 mL bottle) into inlets 5 and 6 marked with dark gray dots.
7. Place the chip into the C_1 Single-Cell Auto Prep System then run the **Prime (1782x/1783x)** script. When the Prime script has finished press **Eject** to remove the primed chip from the instrument. This script takes approximately 10 minutes.

NOTE: The chip may stay in or out of the C_1 Single-Cell Auto Prep System for up to one hour between priming and loading.

Preparing the Cells

1 Prepare a cell suspension of a concentration of 166-250 K/mL in native medium prior to mixing with C_1 suspension reagent and loading onto the chip. This will ensure a total cell count pipetted on chip of approximately 500-750 cells. As few as 200 cells total, from 66 K/mL in native medium, may be loaded on the chip. Fewer cells loaded may yield fewer captured cells. A final volume of 0.5 to 1 mL is desirable so that there are enough cells for both the chip and the tube controls.

NOTE: Cells may be counted by any preferred method. If an established cell counting protocol does not exist, we suggest using the disposable hemocytometer C-Chip by iNCyto. See www.incyto.com for instructions for use.

CAUTION! Vortex the C_1 Suspension Reagent thoroughly prior to use. If C_1 Suspension Reagent contains particulates, ensure they are properly removed by vortexing. DO NOT vortex the cells.

2 Prepare cell mix by combining C_1 Cell Suspension Reagent with cells at a ratio of 4:6. An example can be found in the table below.

NOTE: The volume of Cell Mix may be scaled depending on volume of cells available. A minimum volume of 5 µL of Cell Mix is necessary for the chip. The 4:6 ratio of C_1 Suspension Reagent to cells must be maintained.

Components	Volume
Cells 166-250K/mL	60 µL
C_1 Cell Suspension Reagent (Fluidigm)	40 µL

Table 8 Cell mix

Loading Cells

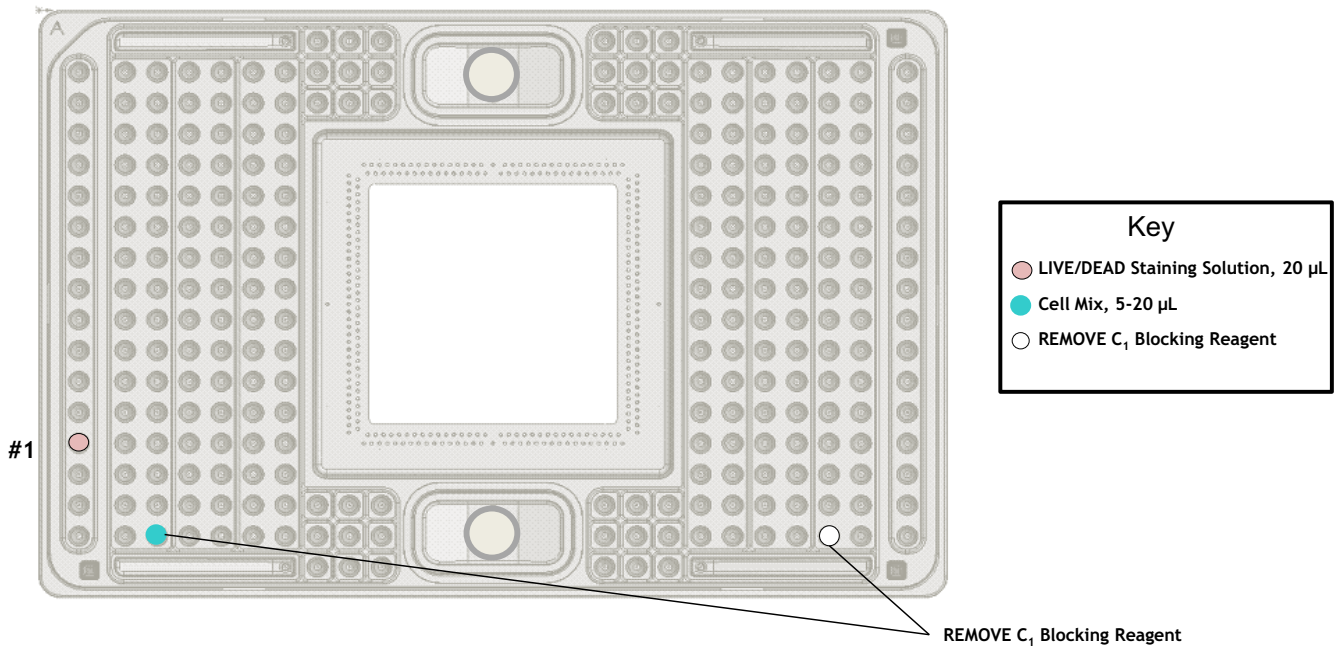


Figure 7 C_1 Chip loading pipetting map

1. Pipette Cell Mix up and down 5-10 times to mix, depending on whether or not the cells tend to clump. Do not vortex the cell mix. Avoid bubbles when mixing as these may cause load failures.
2. Remove blocking solutions from cell inlet and outlet marked with teal and white dots in Figure 7.
3. Pipette 5-20 µL of Cell Mix into the cell inlet marked with the teal dot. Only 5 µL of Cell Mix will enter the chip.
4. Pipette 20 µL of C_1 Live/Dead staining solution into inlet 1 marked with a pink dot.

   If not staining, pipette 20 µL of C_1 Cell Wash Buffer into inlet 1.
5. Place the Chip into the C_1 Single-Cell Auto Prep System; then run the **Cell Load (1782x/1783x)** or **Cell Load & Stain (1782x/1783x)** script. When the script has finished, press **Eject** to remove the chip from the C_1 Single-Cell Auto Prep System.

Optional: Starting the Tube Control: Lysis and Reverse Transcription

If you choose to start tube controls, please see “Appendix 1: Running the Tube Controls” on page 23 for instructions.

Imaging the Cells

Cells may be imaged on a microscope compatible with C_1 chips. The guidelines for the selection of a microscope are outlined in the *Minimum Specifications for Single-Cell Imaging* document, PN 100-5004. Please contact Technical Support for this document or with any questions. Call 1-866-358-4354 (within U.S.) or 1-650-266-6100 (outside U.S.), or email techsupport@fluidigm.com.

Running Lysis, Reverse Transcription, and PreAmp on the C_1 System

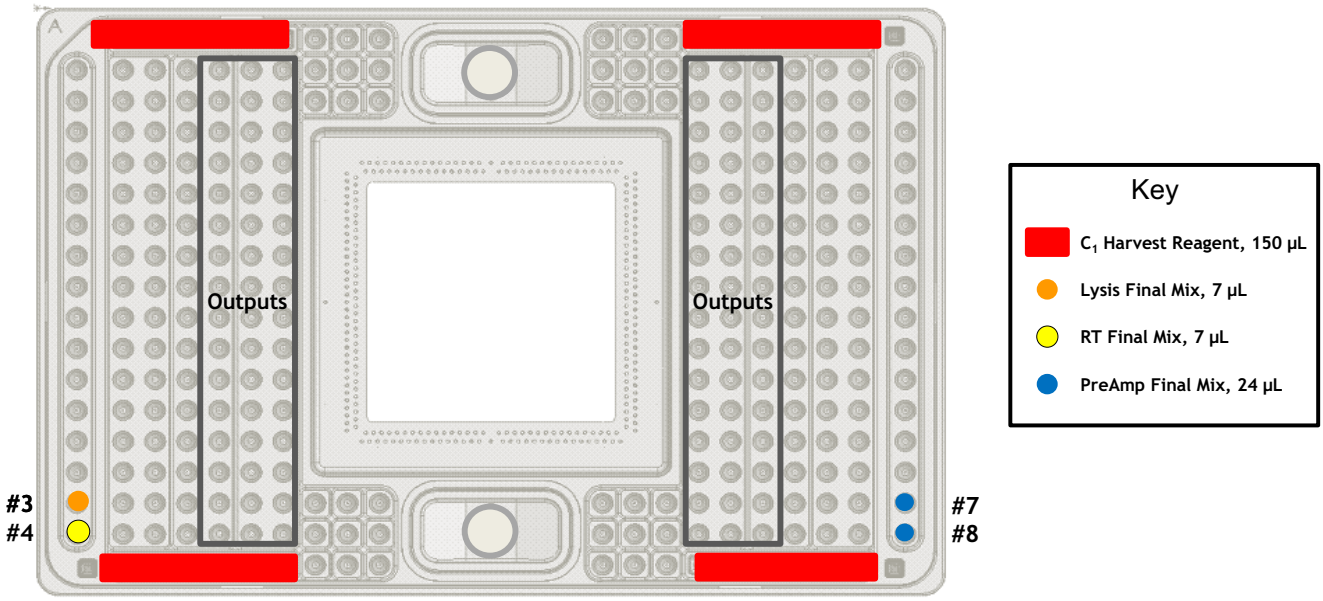
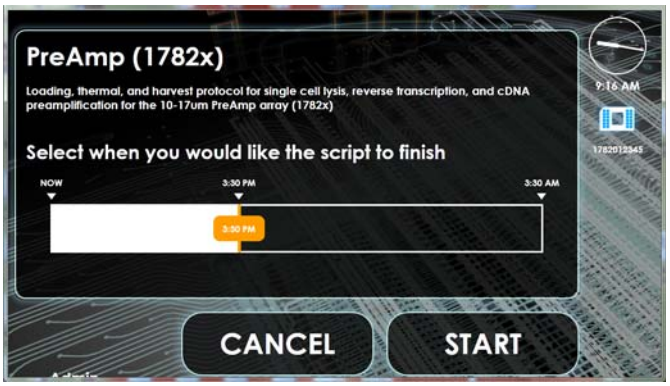


Figure 8 C_1 chip Lysis, RT and PreAmp pipetting map

1. Pipette 150 µL Harvest Reagent into the four reservoirs marked with large solid red rectangles in Figure 8.
2. Pipette 7 µL Lysis Final Mix in well #3 marked with an orange dot.
3. Pipette 7 µL RT Final Mix in well #4 marked with a yellow dot.
4. Pipette 24 µL PreAmp Final Mix in wells #7 and #8 marked with blue dots.
5. Place the Chip into the C_1 Single-Cell Auto Prep System and run the **PreAmp (1782x/1783x)** script.

NOTE: The PreAmp (1782x**/**1783x) script may be run overnight. This protocol takes approximately 6 hours total—4 hours for lysis, reverse transcription, and preamplification, and 2 hours for harvest. This protocol can be programmed to harvest at a time that is convenient to your workflow. For example, the harvest function could be programmed to be complete upon the start of your workday the following morning.



The PreAmp (1782x/1783x) script contains the following thermal cycling protocols:

Reverse Transcription	
Temperature	**Time**
25 ºC	10 min
42 ºC	1 hour
85 ºC	5 min

Preamplification			
Stage	**Temperature**	**Time**	**Cycles**
Enzyme activation/ RT Inactivation	95 ºC	10 min	1
Denature	95 ºC	15 sec	18
Anneal/Extend	60 ºC	4 min	
Hold	4 ºC	hold	

Table 9 Thermal cycling protocols

Optional: Continuing the Tube Control: PreAmp

If you are running tube controls, please see “Appendix 1: Running the Tube Controls” on page 23 for instructions.

Harvesting the Amplified Products

1 When the PreAmp script has finished press **Eject** to remove the chip from the instrument.

NOTE: The chip may remain in the C_1 Single-Cell Auto Prep System for up to one hour after harvest before removing products from their inlets.

2 Transfer the C_1 chip to a post-PCR lab environment.

3 Aliquot 25 µL of C_1 DNA Dilution Reagent into each well of a 96-well plate.

4 Carefully pull back the tape covering the Harvesting inlets of the chip using plastic removal tool.

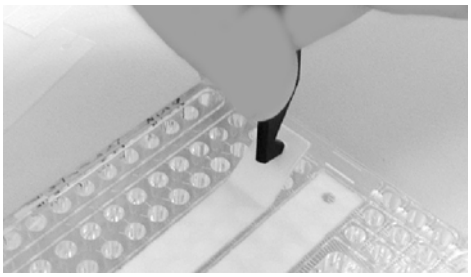


Figure 9 Tape removal

5 Using an 8-channel pipette, pipette 3 µL of the harvested amplicons from the inlets according to the diagram below and place in a 96-well plate.

Components	Volume
C_1 DNA Dilution Reagent (Fluidigm) (30 mL bottle)	25 µL
C_1 harvest amplicons	3 µL

Table 10 Harvest amplicon dilution

NOTE: These preamplified samples are now ready for subsequent analysis on the BioMark following *Fluidigm 96.96 Real-Time PCR Workflow Quick Reference* (PN 68000130) with TaqMan Gene Expression Master Mix (2X, Applied Biosystems, PN 4369016) instead of TaqMan Universal PCR Master Mix (2X, PN 4304437).

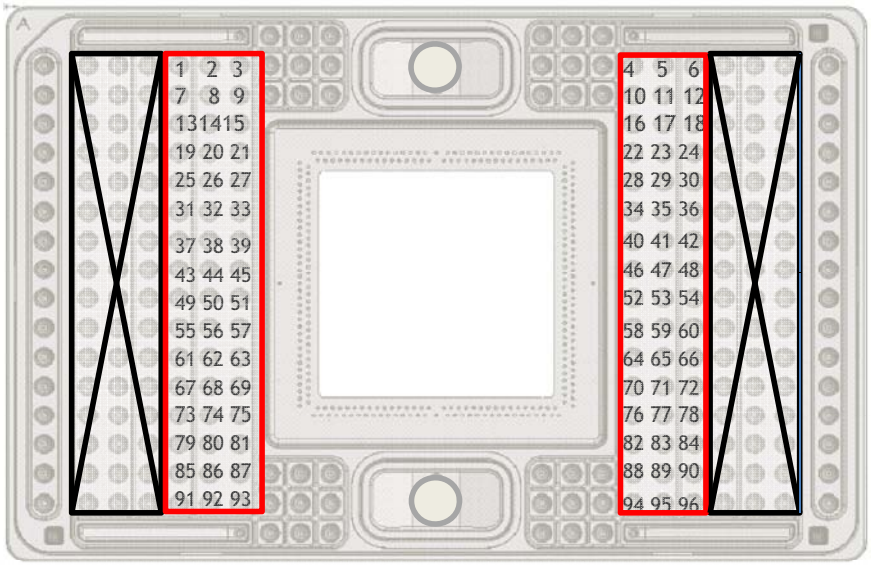


Figure 10 Pipette map of reaction products on the C_1 chip

6 Pipette harvest products out of the left-side wells of the C_1 chip into the 96-well plate as shown in the diagram below.

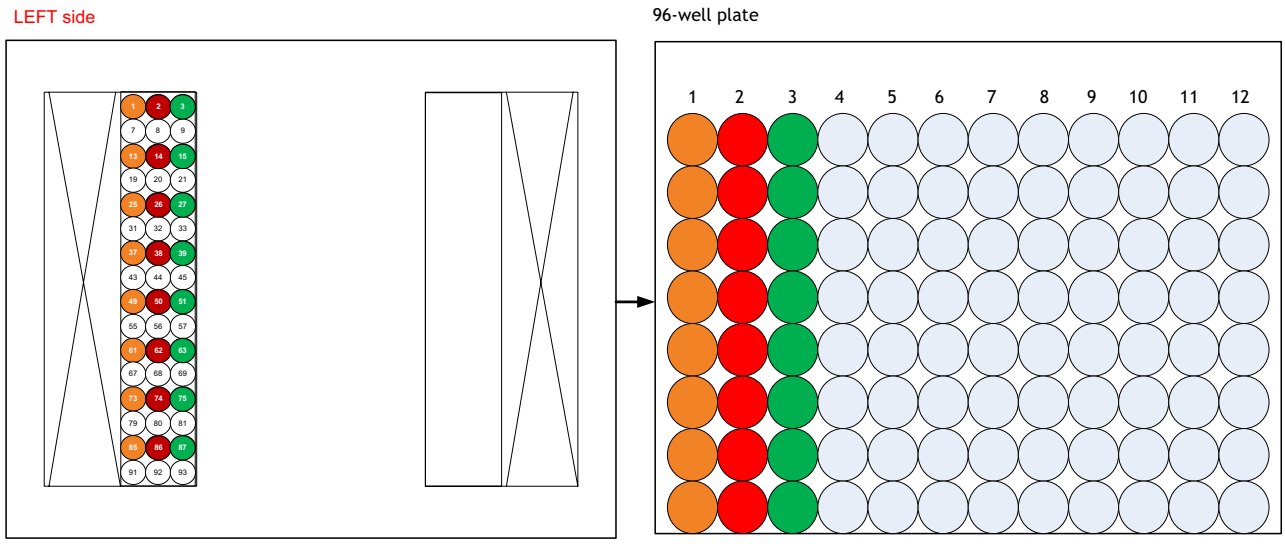


Figure 11 First three harvest product pipette steps

7 Pipette harvest products out of the C_1 chip and into a 96-well plate as shown in the diagram below.

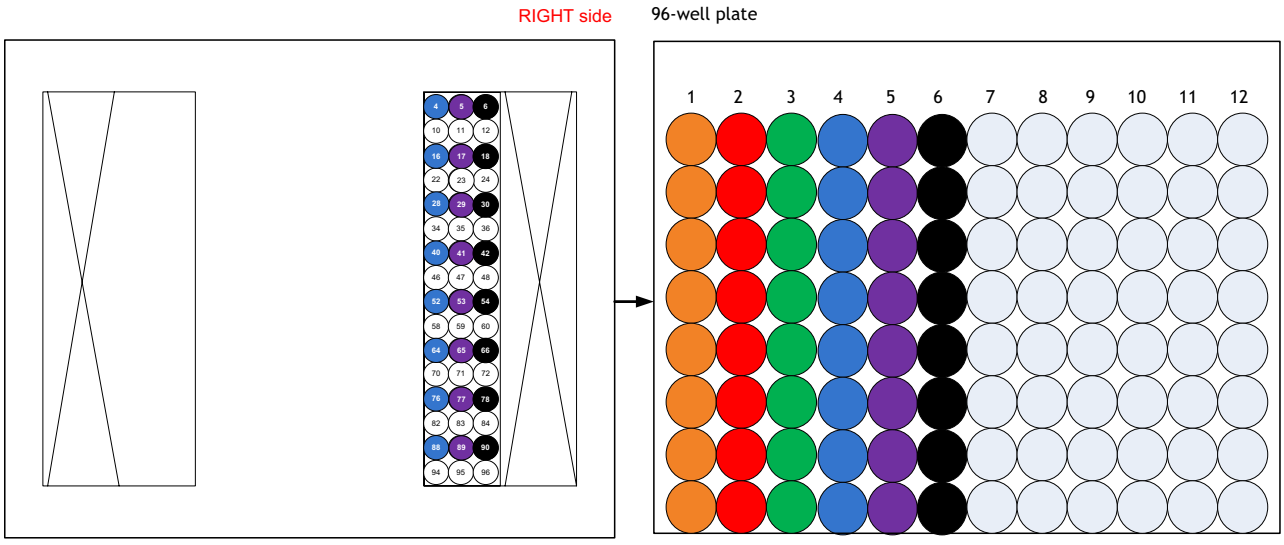


Figure 12 Fourth, fifth and sixth pipetting steps

8 Pipette harvest products out of the C_1 chip and into a 96-well plate as shown in the diagram below.

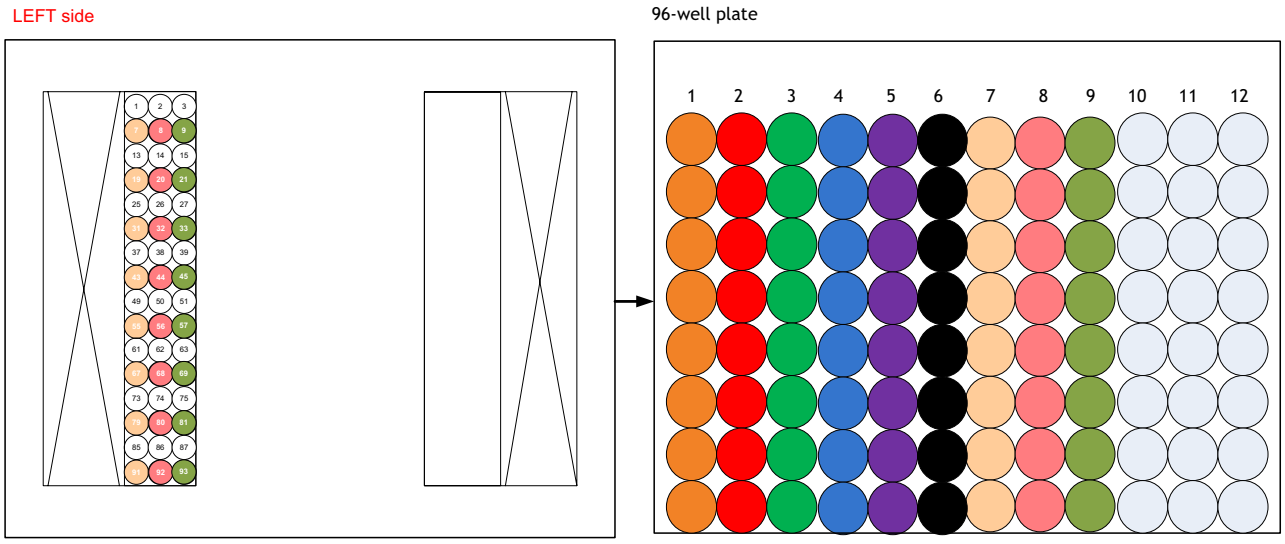


Figure 13 Seventh, eighth and ninth pipetting steps

9 Pipette harvest products out of the C_1 chip and into a 96-well plate as shown in the diagram below.

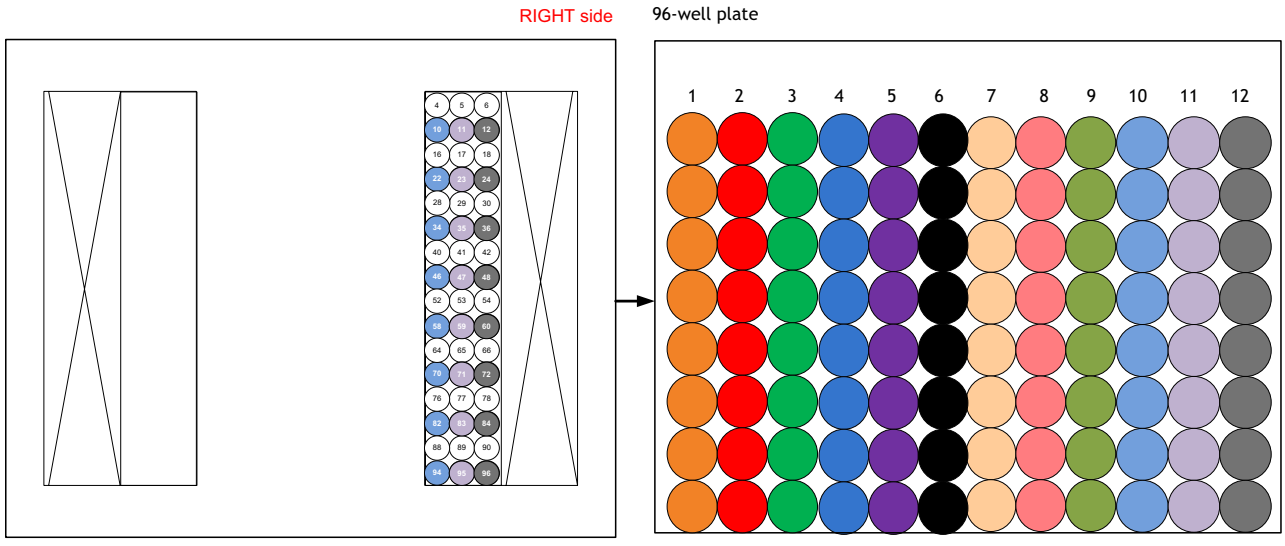


Figure 14 Tenth, eleventh and twelfth pipetting steps

Appendix 1: Running the Tube Controls

Washing the Cells

1. Pellet remaining cells (1 mL volume is easiest). Speeds and durations may vary. We suggest spinning cells at 300G for 5 minutes.
2. Remove buffer from pellet by gently pipetting out the supernatant media without disturbing the cell pellet.
3. Resuspend cells in 1 mL C_1 Cell Wash buffer by pipetting up and down at least 5 times. This is wash 1.
4. Pellet cells again and remove supernatant.
5. Wash a second time by resuspending in 1 mL by pipetting up and down 5 times.
6. Pellet cells a third time and remove supernatant.
7. Resuspend cells in C_1 Cell Wash Buffer approximately 90% original volume, to keep original concentration, assuming a 10% loss.
8. Prepare two tube controls by combining the following:

Tube 1 - Positive Control	Tube 2 - NTC	Volume (µL)
Washed Cells	C_1 Cell Wash Buffer	1.0
Lysis Final Mix	Lysis Final Mix	2.0
		3.0 subtotal
Allow reagents to sit at 25ºC (room temperature) for 5 minutes. Then add to each tube:		
RT Final Mix	RT Final Mix	**2.0**
		5.0 total

Table 11 Tube controls

9. Vortex briefly and spin to collect contents.
10. In a PCR thermal cycler, run the following protocol:

Reverse Transcription	
Temperature	**Time**
25 ºC	600 sec
42 ºC	3,600 sec
85 ºC	300 sec

Table 12 Thermal cycling protocol

11 Once thermal cycle protocol has finished, combine the following in two tubes of an unused PCR strip.

Components	Volume
PreAmp Final Mix	3.33 µL
RT Reaction	0.35 µL

Table 13 PreAmp reaction

12 In a PCR thermal cycler, run the following protocol:

Preamplification			
Stage	**Temperature**	**Time**	**Cycles**
Enzyme activation/ RT Inactivation	95 ºC	600 sec	1
Denature	95 ºC	15 sec	18
Anneal/Extend	60 ºC	240 sec	
Hold	4 ºC	Hold	

Table 14 PreAmp thermal cycling protocol

Dilute Products and Heat Denature the Enzyme

1 Transfer prepared material to Post-PCR lab.

2 Briefly vortex the prepared products and spin to collect content.

3 Combine the following reagents according to the table below.

Components	Volume
C_1 DNA Dilution Reagent (Fluidigm)	99 µL
PreAmp Product	1 µL
Total	100 µL

Table 15 Dilution of PreAmp products

4 In a PCR thermal cycler, run the following protocol:

Denature the Enzyme		
Temperature	**Time**	**Cycles**
95 ºC	10 minutes	1

Table 16 Thermal cycling parameters

5 Once denature is completed, vortex for 5 seconds and spin to collect contents.

6 Store the diluted PreAmp products at -20 °C until use.

Running the Reaction Products on a 96.96 Gene Expression IFC

Follow *Fluidigm 96.96 Real-Time PCR Workflow Quick Reference* (PN 68000130) with TaqMan Gene Expression Master Mix (2X, Applied Biosystems, PN 4369016) instead of TaqMan Universal PCR Master Mix (2X, PN 4304437).

Always handle the reaction products from the C_1 chip in the post-PCR lab.

Appendix 2: Chip Pipetting Map

Overview of chip pipetting

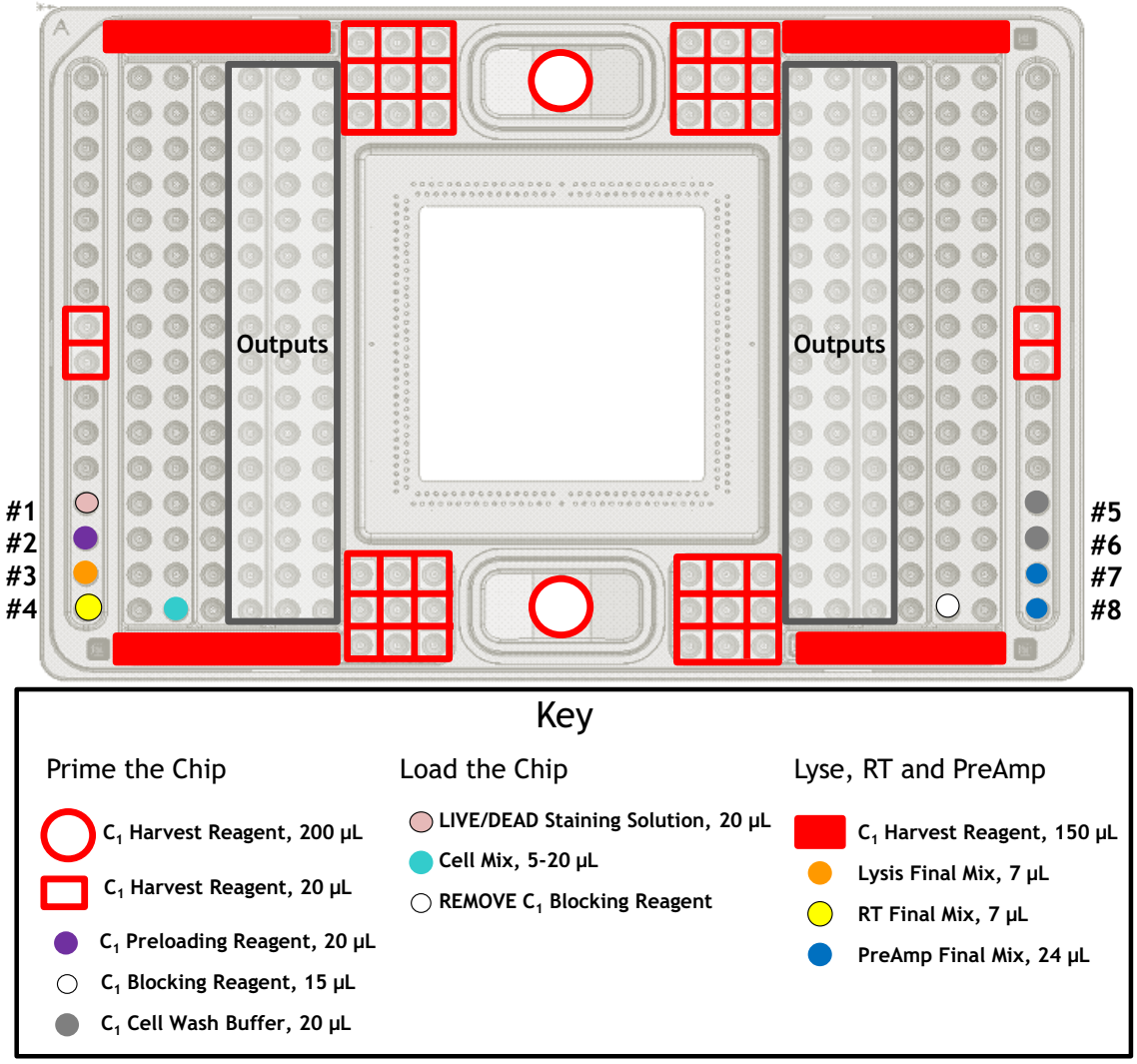


Figure 15 Chip pipetting map

For More Information

To find out more about the information in this or any other Fluidigm Protocol, contact techsupport@fluidigm.com or call:

North America: 1 866 358 4354
Europe: +33 1 60 92 42 40
Japan: +81 3 3555 2351
All other countries: +1 650 266 6100

© 2012, Fluidigm Corporation. All rights reserved. Fluidigm, the Fluidigm logo, BioMark, C_1, Dynamic Array, and DELTAgene are trademarks or registered trademarks of Fluidigm Corporation. All other trademarks are the property of their respective owners. For Research Use Only. Not for use in diagnostic procedures.

NOTICE TO PURCHASER: Disclaimer of License for PreAmp Methods

No right to perform Life Technologies Corporation's patented pre-amplification methods is conveyed with the purchase of the DELTAgene Assay from Fluidigm Corporation. A license to use the product with such pre-amplification methods can be obtained (i) with purchase of a C_1 Single-Cell Auto Prep Array IFC, BMK-M-96.96, or BMK-M-48.48 Dynamic Array IFC from Fluidigm Corporation or (ii) by a separate license from Life Technologies Corporation. No right to resell the products and no other rights (such as real-time PCR methods, apparatus, reagents or software to perform digital PCR methods) are conveyed by Life Technologies Corporation expressly, by implication, or by estoppel. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.